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14. ABSTRACT

We have initiated a long range project aimed at engineering microorganisms with multiple new capacities, using *Escherichia coli* as the prototype organism. Our approach is to determine which organisms can serve as direct donors of genetic material, in that their genes are expressed by the *E. coli* cell, and which require additional engineering. We are developing methods for incorporating up to 300 kb fragments into the *E. coli* chromosome to allow whole pathways to be encoded by foreign DNA. Also, we are looking for dramatic phenotypic differences in *E. coli* generated by cloned fragments from foreign organisms, using the mutator phenotype as an example. We have identified genes from *Lactococcus lactis* and *Pseudomonas aeruginosa* that cause mutator phenotypes when overexpressed in *E. coli* and interestingly, one of these encodes a regulator for multiple drug resistance.

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FINAL REPORT

Grant #: N00014-01-1-0148

PRINCIPAL INVESTIGATOR: Dr. Jeffrey H. Miller

INSTITUTION: University of California, Los Angeles

GRANT TITLE: Syngenomics Applied to the Tryptophan Biosynthetic Pathway

AWARD PERIOD: 13 November 2000 - 30 November 2002

OBJECTIVE: To add new capacities to a standard microorganism, *Escherichia coli* by incorporating genes from different sources.

APPROACH: We have several approaches to reach the first milestone in a long ranging project. The first involves finding out which foreign organisms can express their DNA in *E. coli* without additional genetic engineering. In order to achieve this we decided to use the tryptophan synthetase A gene as a model gene to express and monitor. The second approach is to develop methods to incorporate large segments of foreign DNA into *E. coli*, initially via plasmids and subsequently as part of the chromosome. A third approach is to screen foreign genomes for DNA segments that when expressed in *E. coli* show dramatic effects.

ACCOMPLISHMENTS: First, we tested the expression of tryptophan synthetase A in *E. coli* from different genomic sources by examing cloned DNA fragments. We monitored the ability of each cloned gene to complement a tryptophan synthetase A deficient strain of *E. coli*. The microorganisms that by this and other criteria were able to express their genes with their own promoter operating in *E. coli* were: *Campylobacter jejuni*, *Lactococcus lactis*, *Helicobacter pylori*, and *Pseudomonas aeruginosa*. Those failing to express in *E. coli* under their own promoter include: *Caulobacter acetobutylicum*, *Aquifex aeolicus*, *Bacillus subtilis*, and *Haemophilus influenzae*.

We have completed a bioinformatics study of the existing sequenced microbial genomes, currently 86, including one sequenced in our laboratory. We researched the biosynthetic pathways to generate a database of the potential metabolic engineering pathways compatible with *E. coli*. From this we determined that ultimately, finding ways of incorporating large DNA fragments into *E. coli* is the best way to engineer strains with new multiple capactities. We are therefore

developing methods to generate *E. coli* strains with large inserts of foreign DNA. In addition to chromosome integration systems acquired from other investigators, we have also developed our own plasmid, pHybrid, that is a modified bacterial artificial chromosome vector. This would allow us to incorporate 100-300kb segments of foreign DNA into the chromosome via gene replacement of the 100 genes covered by a viable *gpt-lac* deletion. Initially, we have generated BAC clones from *Lactococcus lactis* genomic DNA (the entire sequence of L. lactis is known) as a proof of principle, with segments of up to 50 kb, sequenced the ends, and are now putting these into the *E. coli* chromosome.

As an additional approach, we screened Sau3A1 partial digest libraries, 3-5 kb in length, cloned into a multicopy vector, for expression in *E. coli* that resulted in dramatic phenotypic differences. We focused initially on those that created mutator effects by using an indicator strain that generates blue papillae in response to frameshift mutators. We examined large numbers of clones generated from the genomic digests of both *Lactococcus lactis* and *Pseudomonas aeruginosa* and found several clones from each organism that produced mutator effects. Sequencing these clones revealed the identity of the genes that increased the *E. coli* mutation rate when overexpressed in *E. coli*. The genes from *Lactococcus lactis* that showed these effects were the *uvrA* gene, *rnhA*, an unassigned open reading frame, and a truncated *dnaA* gene. The clones from *Pseudomonas aeruginosa* that created the mutator phenotype all expressed the *nfxB* gene, a regulator of a multi-drug resistance pathway. This latter result has some provocative implications.

CONCLUSIONS: DNA from a variety of organisms can be expressed in *Escherichia coli*, providing a treasury of genes from which to use to build new capacities for a multi-potent microorganism. Although additional engineering can allow the use of genes and sets of genes from an even wider array of microorganisms, this additional step or steps is not required at the present time. Analysis of pathways from many different sequenced genomes indicates that bringing large fragments of genomes (50-300 kb) into an organism such as *E. coli* is required to provide whole pathways for metabolic engineering. Genes from other organisms can generate interesting phenotypes in *E. coli* that can be used in screening and selection, as was done for mutators arising from inserting *Pseudomonas aeruginosa* and *Lactococcus lactis* DNA. This can be used as a shortcut for finding important genes.

SIGNIFICANCE: Our studies have laid the groundwork for building microorganisms with increased capacities, using *E. coli* as the sample microorganism. Genes from other

microorganisms can express important phenotypes, and methods for incorporating very large fragments can outline a plan for introducing multiple capacities into a single organism. The finding that expressing a regulator of multidrug resistance from another organism, in this case the *nfxB* gene from *Pseudomonas aeruginosa*, creates a mutator phenotype in *Escherichia coli* is tantalizing, since it suggests a relationship between acquisition of drug resistance and the induction of high mutation rates that may have profound implications for antibiotic use and disease control.

PATENT INFORMATION: None

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